Chap. 3

N40679

3. INPUT AND DISPOSITION OF LEAD IN MAMMALIAN SYSTEMS

1. Absorption

a. Skin

The major routes of absorption for lead are generally recognized to be the gastrointestinal tract and the lungs. The third major potential portal of entry is the skin, whose importance in the case of lead is generally considered to be essentially nil. The question of lead absorption through the skin has been investigated. Most of this work was done prior to 1920, at which time the primary concern was with reference to relatively high-level dermal exposure in industrial environments and from application of lead-containing cosmetics and lotions.

These early reports are cited by Cantarow and Trumper (1), who summarize by saying that the consensus among modern authorities (circa 1944) is that dermal absorption of inorganic lead compounds is of little or no practical significance in contributing to lead poisoning. This is not to say that dermal absorption of inorganic lead compounds does not occur. Several references are cited in which evidence is provided for dermal absorption of inorganic lead compounds when mixed with animal fat.

Lead salts can react with fatty acids to form lead soaps, and these soaps are absorbed to varying degrees through the skin. The absorption of lead naphthenate through the skin of the rabbit and man has recently been studied (1a). The formulations used were commercial industrial lubricants containing approximately one per cent lead as the naphthenate soap. Under conditions of repeated application to restricted skin

areas, clear evidence of absorption resulted in the case of the rabbits and less certain but suggestive evidence of absorption was found in the human subjects. It is not certain that lead soaps are absorbed better than inorganic salts. In one study, the concentration of lead in the kidneys of rats was found to be elevated to approximately the same degree following dermal application of lead acetate as following dermal application of lead oleate (1b).

The absorption of organic compounds of lead, notally tetraethyl lead, is far greater, sufficiently so that it is possible to induce signs of acute poisoning following local application.

Although it seems unlikely that dermal absorption of lead is a significant contributor to the total body burden of lead with usual environmental

sources, critical experiments should be conducted to confirm that this is so. It is also possible that a more diligent search of the literature than I was able to undertake would unearth the necessary data.

b. Gastrointestinal tract

Absorption of lead from the gastrointestinal tract has been studied in both man and animals, using both tracer doses of radioactive lead and larger doses of nonradioactive lead. Most such experiments have been conducted using the "balance trial" approach. Dietary intake is increased by a specified amount per day over baseline and the change in urinary and fecal excretion is noted. The change in urinary excretion and body burden is considered to represent net absorption. Utilizing this approach, the net absorption of lead in a small number of human subjects was estimated to be 6-7 percent (2). In these studies, the dose of lead was 0.3-2 mg. Pb/day, which represented increases over the normal dietary intake of 2- to 7-fold. In these studies, the relatively soluble acetate and chloride salts of lead were used. Using tracer doses of ^{212}Pb the absorption of single doses of lead was found to be 16.0, 8.1, and 1.3% in three men (3). The ages of these men were 27, 40 and 59 years respectively. The authors speculate from this single-dose study that the absorption of lead may be age-related. Although a tracer dose of radioactive lead was administered, the actual conditions of absorption were really against a background of normal dietary lead. Some studies have also been made of absorption of lead from the gastrointestinal tract of animals. Apparent or net absorption of lead in sheep is approximately 1.3% over the range of 2-108 mg. Pb/day and is quite similar

in rabbits (4). It would seem from this report that the absorption of lead in herbivorous animals is less than in man and perhaps other omnivorous or carnivorous species. In an experimental situation the absorption of lead in mice is stated to be over 90% using small doses (5).

There appears to be little information concerning the influence of the form in which lead is administered upon the degree of absorption. For the general spectrum of inorganic salts of lead there appears to be no pronounced difference in the lethal dose of lead to calves (6). In these studies, lead was about equally toxic when administered as the acetate, phosphate, oxide, carbonate, and even as dried flakes of paint; Only in the case of metallic lead and lead sulfide was the lethality of lead noticeably less. I am not aware of similar comparisons in other species. The similarity in the lethality of so many salts suggests dietary constituents or the gut mucosa serves as restrictive factors functioning as a common denominator for many forms of lead. It would be of some importance to know if these observations on cattle hold true for other species. It would also be useful to know in what chemical form lead exists in the gastrointestinal tract, ~~and just what~~ the degree of dissociation of lead compounds in the presence of various dietary environments, and the effect of diet on lead absorption

Some attention has been directed toward the effects of calcium, phosphorus, and Vitamin D on lead content of organs in experimental animals; no studies have been done on man and referred to lead arsenate Most of these studies antedate 1940. The general observation is that the amount of lead retained in the body ~~with~~ after oral administration is depressed by high levels of dietary calcium and phosphorus and increased by high

levels of Vitamin D. It is not entirely clear to what degree these effects are the results of influences on lead absorption as opposed to lead deposition in tissues (principally bone, which has been most-studied) or to alterations in the renal clearance of lead. It seems likely that in the case of calcium at least the effect seen is due to interference with the gastrointestinal absorption of lead (7, 8).

This is most readily explained on the basis of competition between calcium and lead for the Vitamin D-controlled transport mechanism involving these two ions. The effect of high phosphorus probably is due to the great insolubility of lead phosphate, and to consequent reduction availability of lead for absorption.

c. Respiratory tract

The entry of lead into the body via the respiratory tract has been the subject of considerable study in recent years, but much remains to be done before a completely satisfactory understanding can emerge. Particles of any substance entering the respiratory system via the nose or mouth are incompletely retained. The percentage retention is dependent mainly on the aerodynamic properties of the particles, as well as the respiratory characteristics particularly the relative units of nose and mouth breathing, the inspiratory and expiratory flow rates and tidal volume. The pattern of deposition within the respiratory tract also varied considerably, depending on the magnitude of these major factors. This is a matter of some importance because lead deposited inthe interior nares is removed by nose blowing and wiping while particles deposited in the nasopharynx are eventually swallowed and probably absorbed to about the same degree as the lead entering the gastrointestinal tract in food and water. Even the lead deposited in the trachea and larger bronchi, unless very soluble, finds its way into the gastrointestinal tract as a result of its proximal movement by ciliary action and coughing. By contrast, lead deposited in the pulmonary alveolar

bed is assumed to be absorbed directly into the blood stream. The interaction and nature of all these factors as they affect the fate of inhaled particles in general has recently been reviewed (9).

The particle size distribution of lead in the atmosphere is of primary importance. Some studies have been reported. Robinson and Ludwig (10) reported on samples taken in Los Angeles, San Francisco, Chicago, Philadelphia, Cincinnati, and rural Oklahoma and Arizona. There was little variation among these widely-scattered places as to mass median diameter (MMD) of the particles. For these locations the average MMD was 0.25μ with 50% of the particles ranging between 0.16 and 0.43μ. In another study the average MMD for Cincinnati was found to be 0.18μ, while in a nearby suburb it was 0.42μ (11). For Cincinnati 75% of the aerosol mass was in particles less than 1μ, while in the suburb it was 65%. Although more extensive studies of this important point are needed, it seems that the major concern should be over the likely fate of lead particles with MMD's in the range of 0.1 to 1.0μ, and that the average MMD in the general atmospheric environment is about 0.25μ.

For inhaled particles, inggeneral, 0.1-1.0μ MMD it is estimated on the basis of the ICRP estimates (9) that, at a tidal volume of 750 ml. and a rate of 15 breathes per minute, the total deposition in the respiratory (passages and in the lungs) is about 20-60% of that inhaled. For particles 0.2-0.5μ MMD pulmonary deposition is about 20-25%, while nasal and tracheo-bronchial deposition is negligible. Between 0.1 and 0.2μ MMD, pulmonary deposition is somewhat higher and between 0.5 and 1.0 it is somewhat lower. Tracheo-

bronchial deposition becomes significant (greater than 5%) for particles smaller than 0.1μ MMD and nasal deposition becomes significant for particles greater than 0.5μ MMD. Thus, total retention of inhaled lead can reasonably be expected to be about 25% and actual pulmonary retention (as distinguished from tracheo-bronchial and nasal retention) about 20%.

Some data are available regarding retention of lead in man. Probably the most thorough study is reported by Nozaki (12). In this study a distinction was drawn between upper respiratory and lower respiratory retention. This distinction was based on the point of inflection* in the CO_2 concentration curve of exhaled air. Lower tract retention presumable represents predominantly pulmonary retention probably with a certain amount of tracheo-bronchial retention, while upper tract retention represents nasal and tracheo-bronchial retention. At any rate, at 10 rpm and tidal volume of 1350 cc, and going from 0.1μ up to 1μ (definition of diameter not specified)), total retention increased progressively from 39% to 63%. Over this range lower tract retention increased progressively from 25% to 36%, with a slight dip to 22% at 0.2μ. The trend toward increasing retention in the lower tract with increasing particle size does not conform to theoretical expectation (9), but the general degree of retention is only slightly greater than would be expected. By contrast, upper tract retention is considerably higher than would be expected. Noazki found that more shallow breathing at the same minute volume greatly decreased upper tract retention but had no significant effect on lower tract retention.

In another study in man utilizing ^{212}Pb adsorbed to aerosol particles

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* The point at which the CO_2 concentration rises sharply probably serves as a marker separating tracheo-bronchial air from alveolar air.

having a diameter of 0.2μ, average lung deposition was 25% with a range of 14-45% (13). This is very much in line with theoretical expectation, Evidently, little of the lead retained found its way down the digestive tract since fecal excretion of the inhaled radio activity over 2-3 days was only 0.3-8.4% of the inhaled dose.

Other studies in man have been conducted but they are less useful in the context of the general environmental situation. Kehoe, for example, studied lead disposition in man working with very small (90% = 0.01-0.1μ) and rather large (50% > 0.9μ) particles of lead oxide generated by burning tetraethyl lead in propane (2). The major value of Kehoe's study is the fact that he was able to determine the degree to which the lead which was deposited found its way into the gastrointestinal tract. Lung deposition was high both when the particle size was small (36%) and when it was large (46%). These results are consonant with theoretical expectation, The balance studies revealed that when the particle size was very small, virtually all of the lead retained in the lung remained there, and while when the particle size was large, about 40% of the lead retained found its was into the gastrointestinal tract.

In a study of the deposition of inhaled lead in both occupational and urban settings, Mehani (14) found 37-47% deposition,,which is almost identical to Kehoe's results and rather similar to those of Nozaki (2). Unfortunately, particle size distribution was not determined in Mehani's study. Horiuchi

et al (15) subjected men to daily aerosol inhalations of lead acetate. Particle size distribution was stated to be: 67% < 1μ and 95%+ < 5μ. Balance studies in these people were conducted, as in the case of Kehoe's studies. Lung ~~retention~~ deposition was 49-52%. Much of this was eventually excreted in the feces, as might be expected from the relatively large particle size distribution. The particles were stated to be in the form of an aqueous mist of lead acetate solution.

This section, dealing with lung ~~retention~~ deposition, would be incomplete without some consideration of the fate of the lead which is deposited in the pulmonary bed. There is very little information available on that point. Hursh et al (13) estimated lung clearance to be $T_{\frac{1}{2}}$ = 6.5 hours. They were working with tracer doses of ^{212}Pb ~~adsorbed to~~ in water vapor and it is questionable whether their observations would be valid for carrier amounts of the lead compounds normally found in the atmosphere. Schroeder and Tipton in a study of the lead content of tissues found an age-related increase in the concentration of lead in the lung of Americans, but not in people from other lands. This finding cannot be interpreted as meaning that lung clearance is so slow as to result in accumulation over the years, since the accumulation could be due to movement into the lungs from the systemic circulation. Einbrodt et al (1968) found an actual decrease in the lead burden of the lungs with increasing age in a series of German women. It is not possible to say whether differences in patterns of age-related accumulation, U.S. vs. foreign, reflect differences in lung clearance due to differences in the physical and chemical characteristics of the inhaled particles or whether other factors are operative.

The Task Force Group on Lung Dynamics classifies lead sulfide (12) and lead halides in the group of metal salts exhibiting intermediate clearance rates (weeks), without specification as to the basis. Other salts of lead are not classified.

There clearly is a need for long-term study of the lung clearance of lead. This is so because short-term determination of lung clearance does not necessarily serve to describe total clearance. As a matter of fact, for insoluble particles a slow phase of clearance is noted with half times of approximately 120 days and of 1 and 4 years for plutonium and thorium.

2. Distribution

In reviewing the subject of the distribution of lead in the body, it might be useful to establish first what the important considerations are. First, it seems important to distinguish between body distribution seen at or near steady-state conditions and distribution seen shortly after intake. The shifting patterns of distribution with time following a single dose of lead or during a period of continuing intake reflect differences in inherent affinity on the one hand and accessibility as determined by minute volume of blood flow on the other. Second, it is important for the purposes of this panel study to cite data relating the magnitude of the lead burden in various organs and tissues to the concentration in the accessible body fluids -- principally blood and urine. This is so because the toxic effects of lead, as documented in the literature, seldom (if ever) are correlated with concentrations of lead in the target organs per se.

It has long been known that lead is a bone-seeker and that under conditions of continuing intake, and even shortly after single large doses of lead, the amount of lead in the skeleton far exceeds the amount in all the other tissues of the body combined. In the most extensive study of the distribution of lead in man, the skeletal burden was estimated to be 91% of the total body burden under conditions of average environmental exposure (16). The concentration of lead in various bones of the body is quite variable on a fresh organ weight basis. However, when the data are recalculated on the basis of concentration in bone ash the differences become insignificant (17). Beyond that, the distribution of lead in the

body under conditions approaching the steady state is reasonably uniform. On a wet weight basis no other organ approaches bone as to concentration of lead. However, on the basis of ash weight, a number of organs, including the aorta, liver, and kidney, have higher concentrations of lead than bone. Similar exhaustive analyses of the distribution of lead in animals under steady state conditions are not available, so far as I know at least.

The interrelationship of the concentration of lead among the organs of the body has been the subject of limited study. In a study of the kinetics of the disappearance of lead from various organs following administration of a tracer dose of ^{210}Pb, there was considerable variation in the rate of disappearance even eight days later (18). Similarly, in a survey of the lead content of organs in man, it was found that, over the normal life span, organs and systems exhibit different characteristics with reference to age-related patterns of lead accumulation. (16) This being the case, it is hardly to be expected that the concentration of lead in the blood or in the urine can be arithmetically related to concentrations elsewhere in any simple fashion.

Early studies of lead metabolism in man were largely concerned with total balance. In his detailed studies of lead metabolism in man, Kehoe measured the dietary intake and outgo of lead under normal conditions and with small daily oral doses of lead acetate. He concluded that the average American adult is essentially in balance (19, 20). Actually, his observations concerning the excretion of small daily doses of lead administered for as long as four years indicate a continuing accumulation

in the body (2). It seems unlikely that a different set of laws of kinetics should govern the metabolism of lead at a daily dietary intake level of 0.3 mg as governs the metabolism of lead at an intake level of 1 mg or 2 mg per day. As a matter of fact, in a study of lead metabolism in the rat, there was no apparent difference between the tissue distribution and excretion of tracer (∿ 0.04 mg/kg) and carrier doses (7 mg/kg) of lead (21). Further, the accumulation of lead in bone and other tissues with age has been documented for man (16). The pattern of accumulation varied from one tissue to another, but for some it appeared to extend almost throughout the lifespan (aorta) and for others accumulation ceased in middle age (bone, liver). Another study also reports increasing concentrations of lead in bone with age, up to about age 40 and perhaps beyond (22).

It is possible that upon reaching a critical concentration in bone, lead accelerates the process of bone resorption, thereby decreasing lead retention. Continuing accumulation also may cause reduced hemoglobin synthesis in old people.

There is considerable uncertainty as to how the rate of loss of lead from the whole body or from any one tissue can best be described kinetically. For most foreign substances introduced into the body, the disappearance rate is proportional to the amount present at any instant in time.

Thus, $A_t = A_o \cdot e^{-kt}$ (1)

where A_t = amount in the tissue at t

A_o = amount in the tissue at time 0

k = rate constant

The important point about this kind of situation is that with continuing intake at a constant rate such a substance should ultimately reach virtual

equilibrium in the body as long as affinity doesn't change. Mathematically the accumulation is described thus:

$$A_t = \frac{A}{k}(1-e^{-kt}) \qquad (2)$$

where A = amount assimilated per unit t and A_t and k are as defined above. The rate of disappearance of alkali earth metals from the skeleton cannot be described adequately using simple exponentials as above. Rather, disappearance is best described as a power function which, graphically, fits a linear log-log plot of fraction retained vs. time. In essence, this amounts to saying that with time the availability of the substance for excretion becomes progressively smaller. Such a model for rate of loss is consistent with accumulation at a rate greater than described in (2). Data for the rate of disappearance of single doses of lead from the body appear to conform to power function kinetics for rat and rabbit (23, 24) and dog (25). The above-cited studies were conducted using tracer doses of ^{210}Pb. If power function models are appropriate for the description of the disappearance of lead from the body, an expression of the biological half-life for lead in bone or in the whole body becomes impossible, in conventional terms at least.

It is possible that the lead ion becomes progressively less available for exchange and excretion by virtue of its ability to act as a nucleating agent for the precipitation of calcium phosphate, trapping the lead in an insoluble matrix (28a). This phenomenon may also explain the increased thickness and density of cortical bone in lead poisoning reported in sheep (28b) and the increased density of trabecular bone in infants (see Chisolm).

Figures are available purporting to approximate the biological half-life of lead in the body. These values are calculated on the basis that lead leaves the body in a manner which can be described by a single

exponential function. This hardly seems realistic in view of the direct evidence cited earlier showing that in experimental animals, in which the amount of tracer in the body is known, a power function is more appropriate. In the procedure described by Holtzmen, the biological half-life is calculated using the equation (26):

$$T_b = \frac{.693\ mC}{If_w} \qquad (3)$$

where T_b = biological $T_{\frac{1}{2}}$

m = body mass

C = lead concentration in body

I = intake/day

f_w = fraction of I retained

This sort of procedure can only yield crude "ball park" figures since C is quite variable among individuals and since the elements entering into the calculation of f_w (such as transfer from gut to blood ~~and from~~ air to lung and lung to blood and variable) are poorly defined. Nevertheless, the calculations do express the justifiable belief that the biological $T_{\frac{1}{2}}$ for lead is long. Making all the necessary assumptions about C and f_w, Holtzman comes up with T_b = 2085 days for whole body and T_b = 6350 days for the skeleton. In the case of radium (^{226}Ra) there is some evidence suggesting that the single exponent approach applies to description of whole-body disappearance in man, at least when the rate of loss is measured many years after acquisition of the burden is terminated. The greatest need is for data from infants and old people.

In a series of 4 former radium dial painters, whole-body burden was estimated by direct measurement of body radiation 40 years after exposure (27). The fraction of the body burden being excreted per day was found to be not significantly different from the average of ten cases in which excretion was measured only twenty years following exposure. A single exponent may similarly describe adequately the rate of loss from the body of an old lead burden (which is essentially all buried in bone) but would not apply to the total body kinetics involved in continuing exposure to lead. Even in regard to bone, the concept of a single kinetic pool is not realistic. The rate of loss of lead from the skull of the baboon has recently been reported to be best expressed by a power function model over the initial two years following lead administration (27a).

Another approach to the calculation of biological half-life applies specifically to bone (28). It proceeds on the assumption that

loss of lead from bone occurs only as a result of dissolution of bone during Haversian system remodeling. Using this approach the estimated biological half-life of lead in bone was estimated to be 4620 days for the dog.

While most evidence currently available indicates that the concentration of lead in some organs, notably bone, increases with age in man as a result of continued low-level exposure, there is need for further confirmation and expansion of knowledge in this area. For one thing, there is need for a substantial increase in the number of autopsy specimens analyzed to better define the age-relatedness of lead concentrations in human tissue. It would also be of interest to determine whether the same situation exists in animals and to determine whether dietary factors influence patterns of accumulation. It is of interest to note that Schroeder found evidence for age-related accumulation of lead among non-Americans only in the case of bone and aorta (16). The significance of this observation is obscure, but it might be related to the influence of different background levels of other trace minerals.

The toxicological significance of various concentrations of lead in tissues is poorly understood. In the case of bone it is generally considered that the continuing accumulation of lead consists largely of an inert, toxicologically insignificant pool, which becomes progressively less accessible and exchangeable with time. This is manifested in several ways, among them by the decreasing availability for chelation by EDTA (21). Little is known about the changing availability or exchangeability of lead in other tissues as the body burden ages.

subject of some controversy. Lead added to erythrocytes *in vitro* can be only slowly desorbed with EDTA. Yet, the association with the erythrocyte can be prevented by the prior addition of EDTA. This leads to the conclusion that lead added *in vitro* is associated with the cell surface as a coagulate of a lead phosphate sol. (29). The added lead could in fact, be completely removed from the cells by dialysis with EDTA for 24 hours. Interestingly and significantly, the lead which had been in the cells at the time the blood was drawn could not be removed by this procedure. The authors interpreted this to mean that lead in erythrocytes acquired *in vivo* under normal circumstances is located intracellularly. The important point is that it is probably only slowly exchangeable. In cases of lead poisoning treated with EDTA there is a modest and fairly prompt desorption, but the desorption is far more pronounced when treatment is instituted with dimercaprol (30). This observation is suggestive of an intracellular localization of lead. The studies of Clarkson and Kench cited above (29) suggest only slow exchangeability of lead incorporated into erythrocytes *in vivo*. Little information is available concerning how well the concentration of lead in erythrocytes correlates with the concentration in other tissues. From limited studies, the correlation appears poor (21). With the intravenous administration of a single dose of ^{210}Pb (7 mg Pb/kg), the fraction of the body burden of ^{210}Pb in the blood decreases at a very rapid rate during the first week. It then remains essentially unchanged for the succeeding eight weeks. The fraction of the body burden in the various soft tissues, on the other

Of all the soft tissues in the body, blood has been most carefully scrutinized with regard to its interaction with lead. The concentration of lead in peripheral blood is heavily relied on as an index of lead exposure. Yet there is today no mathematical definition of the relationship between the concentration of lead in blood and the concentration or amount in the total body or in any anatomic or metabolic compartment. It is interesting to note that when small daily doses of lead are administered to man, the concentration of lead in the blood rises sharply but tends to level off fairly early, while the total body burden is still increasing at a ~~substantial~~ rate related to dosage (2). The same is true of the concentration of lead in the urine as in the case of lead in the blood. One is tempted to interpret this as indicating that the concentration of lead, in the blood(and in the urine), is behaving in accordance with first order kinetics (equation 2 would apply) and is proportional to the exchangeable (and hence excretable) pool of lead in the body. If this were so, the lead in blood should be relatively mobile and, therefore, relatively easily desorbed by dialysis or by comparable means.

Graph?

It is common knowledge that more than 95 per cent of the lead circulating in blood is associated with the cellular elements. More specifically, it is associated with erythrocytes.

The manner in which lead is associated with the erythrocytes is still the

hand, decreases throughout the nine-week period, and the fraction of the body burden in the skeleton increased correspondingly. It is possible that a major fraction of the lead associated with circulating erythrocytes is incorporated during cell formation. It is interesting to note in that regard that the concentration of lead in the bone marrow of men exposed to relatively high concentrations of lead was 60 - 184 times greater than the concentration of lead in peripheral blood (31). The opportunity for high uptake of lead during cell formation certainly is present.

The localization of lead in other cells of the body has been studied to some extent. In one study, separation of cell constituents (liver and kidney) by ultracentrifugation was utilized. Distribution to all subfractions was noted (32). It is not likely that these results were a consequence of redistribution during preparation of the subfractions since the pattern of subcellular distribution changed over a period of weeks following administration to the animals. In another study, histoautoradiographic techniques were used. Intracellular localization of lead was claimed (33). While I have seen only the Kettering abstract of this paper, I must admit to grave suspicions as to the possibility of distinguishing intracellular localization of ^{210}Pb autoradiographically.

Translation requested

The distribution of lead to hair has drawn some attention lately because the concentration of lead is almost as high as it is in bone (34). Further, it has been shown to increase appreciably in some cases of lead poisoning (34, 35). Experimental studies in rabbits indicate that there

is a rough correlation between the concentration of lead in hair and in bone (36). It may seem questionable whether this is a promising avenue for the attainment of any precise information concerning concentration of lead in any organs or systems of major toxicological interest. All of the investigators whose work is cited above seemed to have the problem of substantial variability among subjects of similar exposure history. However, since seeming variability often masks precise hidden relationships, the subject merits further study.

3. Excretion

The major routes of lead excretion are generally assumed to be the gastrointestinal tract and the kidneys. This assumption is probably valid for man since the recovery of lead in urine and feces comes close to accounting for the input in balance studies such as have been conducted by Kehoe.

This presumption may not be valid. The excretion of lead in sweat may be of the same order of magnitude as in urine. This possibility was raised based on the amount of lead excreted in the axillary sweat of mean with lead poisoning (36a).

For animals, it is quite likely that the loss of lead from the body by the shedding of hair may constitute a significant source of "excretion". Kopito et al (35) cite one reference indicating that the amount of lead lost in the hair coat may be very substantial. Excretion of lead in milk has been studied with reference to cows (37). This has been of special interest because of the possibility of transferring potentially toxic amounts of lead to infants and children. The concentration of lead in milk is linearly related to the concentration in the blood. Under usual conditions of exposure of cows to lead, this amounts to approximately 10 µg/l. ~~0.01µg/ml~~.

The relative contributions of the gastrointestinal tract and of the kidneys to the overall excretion of lead appears to vary appreciably among

the species studied. In man, urinary excretion appears to predominate over fecal excretion. Following the intravenous administration of ^{212}Pb to two men, the average urinary excretion in the succeeding 24 hours was 4.15% of the dose, with the excretion being virtually terminated in that period (3). During the 48 hours following administration, only 0.27% of the dose appeared in the feces.

In the baboon, the ratio of daily urinary to fecal excretion of parenterally-administered ^{210}Pb ranged between one and two for the period of 12 to 5 days following administration (37a).

In the dog, fecal excretion of parenterally-administered lead has been found to account for less than 1% of the total urinary and fecal excretion (25).

These conclusions are of questionable validity since they were based on the analysis of urine and feces collected udring three-day period only at intervals of two months.

By contrast, in the rat fecal excretion predominates both early and late after parenteral administration of lead (38, 39). In the sheep, the predominance of fecal excretion over urinary excretion is even more pronounced (40). The mechanism of gastrointestinal excretion is predominantly by way of the bile, at least in the rat and sheep (38, 40).

The mechanism of urinary excretion of lead has been studied in man and dog (41) and in the chicken (42). In man and dog, the rate of lead excretion parallels ~~the~~ changes in ~~G.F.R.~~ the glomerular filtration rate, and extrapolates to zero excretion at zero ~~G.F.R.~~ rate, suggesting that movement of lead across the renal tubule contribute to total urinary excretion. At higher levels of lead excretion, excretion was somewhat less in relation to ~~G.F.R.~~ glomerular filtration rate than at low levels, suggesting some degree of tubular reabsorption. On the other hand, studies of the transport mechanisms in chickens suggest that lead is secreted by the renal tubules.

The excretion of lead in rats has been found to be markedly decreased in both the feces and the urine as a result of increasing the ambient temperature from 70° to 89° (43). The reason for this remains obscure, as far as I know. The effect is associated with a markedly higher concentration of lead in the blood at 89° than at 70°.

4. Estimation of Body Burden

This section will include V, 5, "Non-Toxic" Effects, since actions of lead which are not overtly harmful and which occur at low levels of lead exposure are used to estimate the magnitude of a potentially toxic pool of lead -- one which exhibits biological activity.

There are four general kinds of body burden estimates, which differ as to approach and as to the implications of the measurements. They are:

1. Whole body burden
2. Circulating burden (essentially blood lead levels)
3. Mobile burden (amount of lead excreted in response to chelating agents)
4. Biologically-active burden (reflections of disturbances in heme synthesis and renal function)

a. Whole body burden

The only certain way of determining the whole body burden of lead is to ash or wet-oxidize the whole subject and to perform an analysis for lead on a suitable sample. This approach has certain limitations, some of which are quite obvious. First, the information gained would be of little use to the subject himself. Second, the simple mechanics of

reducing a whole human cadaver to ashes or to a solution of inorganic salts makes the procedure ~~totally impractical~~ difficult. As a matter of fact, experimental studies of lead metabolism even in rats seldom are done in this manner. Too often inferences are drawn concerning the amount of lead in a whole animal or in an organ or system on the basis of the amount in a sample of tissue. This is a particularly questionable practice in the case of bone. The only alternative in sight is determination of the whole body radioactivity due to decay of ^{210}Pb.

An accurate knowledge of the whole body burden of lead would be of very limited value unless performed in conjunction with other measurements which would indicate something about the compartmental distribution of the body burden. This is because probably only a small fraction of the total body burden is biologically active. Most of the body burden is sequestered away from the general circulation in the matrix of bone, where it resides as a separate biologically inactive pool bearing no fixed quantitative relation to the far more hazardous small fraction elsewhere in the body. Assuming a total body burden of 120 mg. lead, a subject having 100 mg. in his skeleton will have twice as much lead in his soft tissues and body fluids than one having 110 mg. in his skeleton. Similarly, a skeletal burden of 100 mg. acquired over ten years is probably more mobile than one acquired over thirty years. For these reasons (and probably for others) it is desirable that other procedures be available which measure the amount of lead in sub-compartments.

b. Circulating burden

The concentration of lead in whole blood has long been used as an index of potentially hazardous lead exposure in industry and as a diagnostic

aid in clinical medicine. There is little knowledge of what such analytical results mean in terms of the magnitude of the whole body burden or of the burden in specific organs and systems. Following single doses of lead in rats, there is no correlation between the amount of lead in the blood and the amount in the whole body or in the bones or soft tissues (ED), at least for a period of nine weeks following administration (21). For that matter, using these data, I have not been able to make any meaningful correlations with the amount of lead in any specific organs. It may be that meaningful correlations are possible under conditions of long-term intake and relatively constant rate of intake. To my knowledge, no such effort has been made either in animals or in the limited number of human subjects (impending death)where this might be possible.

The correlation between the concentration of lead in the blood and the magnitude of the "toxic" pool of lead in the body is very difficult to define because of difficulties with the word "toxic". There is no quantifiable "toxic"pool of lead which has ever been defined. There certainly are degrees of toxicity, but these don't correlate very well with concentrations of lead in blood. In acute lead poisoning in cattle, marked signs of illness occur with blood lead levels as low as 0.35 ppm mcg/ml (44). Yet, cattle have been fed lead in small daily doses to the point of maintaining concentrations of lead in the blood well in excess of 1.0 ppm mcg/ml for many months without apparent ill-effects (45). It may well be that animals acquire a tolerance to lead. Some evidence has been presented recently showing a tolerance phenomenon in mice (46). I know of only one report in which any effort was made to correlate ~~degrees of lead intoxi-~~ the severity of clinical symptomatology

~~action~~ in man (or in animals for that matter) with biochemical parameters be known to/altered in association with lead poisoning. Unfortunately, the concentration of lead in the blood was not one of the biochemical ~~parameters~~ measurements ~~used~~ made (47). Information is scarce concerning the correspondence between the severity of clinical signs and the concentration of lead in the blood.

The question of what constitutes a minimal concentration of lead in blood which is associated with signs of lead toxicity seems far from being settled. R. A. Kehoe has insisted repeatedly that this minimal concentration in blood is 0.8 mcg/gm blood. Yet, one finds in the literature reference to levels as low as 0.4 mcg/gm (48) and 0.21 mcg/gm (49). It is possible as no one else has reported similar low values that these values are spurious. After all, how is the judgment to be made as to whether the colic, irritability, or other sign ~~whatever~~ which one sees is to be considered due to lead? The closer a blood level approaches the normal range, the more difficult that problem becomes. This is all aside from the problem of analytical error which ~~will be~~ is considered elsewhere. ~~by Mr. Keenan.~~

c. Mobile burden

The use of chelating agents as aids in the diagnosis of lead poisoning to determine prior high exposure to lead is fairly commonly reported. The chelating agents most commonly used for this purpose are EDTA and penicillamine. There is little question as to the sensitivity of the procedure for distinguishing between average and high lead exposure. The most striking example of this is the report detailing studies on nephropathy in middle-aged people due to childhood lead intoxication (50). It was not possible to distinguish this group from controls on the basis of the rate of spontaneous urinary lead excretion. Yet, with relatively few exceptions

the elevation of lead excretion following administration of EDTA was appreciably higher in the exposed group than in the presumably non-exposed group. It is to be presumed that the two groups did not differ significantly as to lead exposure subsequent to childhood. Penicillamine has also been studied as a chelator for estimating the amount of mobile lead in the body (47). In this study other parameters of exposure, eg. ALA excretion and coproporphyrin excretion, were measured. Studies in animals to determine the source of the lead mobilized by these drugs have been few. One group of investigators concluded from their data that the source of lead was the soft tissues, using EDTA in rats (51). Other studies flatly contradict this conclusion in rats and in rabbits (52, 53, 21). The skeleton is a far greater source of lead with EDTA mobilization than the soft tissues. As a matter of fact, more lead can be mobilized from the body with EDTA than exists in the total soft tissues prior to chelation. The amount of lead mobilized and excreted in response to a standard dose of EDTA in the rat does not accurately reflect the size of the total body burden of lead because the fraction of the total body burden which is excreted varies appreciably with the recency of acquisition of the lead (21). Furthermore, the amount of lead excreted does not accurately reflect the size of the soft tissue burden either, since the mobility of lead in the soft tissues also decreases with time following acquisition. The magnitude of lead mobilization does not correspond well to the amount of lead circulating in the blood either (21).

However there was a correlation between lead mobilization with penicillamine and the level of ALA and coproporphyrin excretion.

d. Biologically-active burden

There are a number of measurable biochemical abnormalities which accompany excessive exposure to lead. Some of these have attracted the attention of industrial hygienists. It has been hoped that these alterations would prove useful as preclinical sensors to warn of impending hazards due to excessive exposure to lead. Most of the attention has centered around several manifestations of the well-known inhibition of heme synthesis by lead.

Several major alterations in heme synthesis are readily measurable in cases of excessive lead exposure in both man and animals. The ones which have received the greatest attention are 1) elevated excretion of coproporphyrin in the urine, 2) elevated excretion of aminolevulinic acid (ALA) in the urine and 3) inhibition of ALA dehydrase activity in circulating erythrocytes. The quantitative relationships of these abnormalities to the body burden of lead have not been well defined. These reflections of abnormal heme synthesis do nevertheless seem to differ as to the sensitivity and precision with which they correlate with certain other parameters of lead exposure. In a study of men ~~industrially~~ occupationally-exposed to lead, the amount of ALA excreted per gram creatinine correlated much better with the ~~degree of intoxication~~ clinical poisoning than did the amount of coproporphyrin excreted (47). The lack of correlation between symptoms and urinary output of coproporphyrin has been reported by others. It is interesting to note that the correlation between ALA excretion and the amount of lead mobilized with a standard dose of penicillamine also was extremely good (r = .92).

In a later report, these same authors correlated ALA excretion with the concentration of lead in the blood (54). The scatter of points around the curvilinear regression line was quite substantial, in contrast to the scatter of points around the linear regression line relating ALA excretion to lead mobilization with penicillamine. These workers also noted that the concentration of lead in the blood was lower for a given level of ALA excretion among workers periodically rotated away from high exposure work areas than it was among workers continually exposed to high levels of lead. They interpreted this to mean that elevated ALA excretion persists longer than elevation of lead in the blood.

The accumulation of ALA resulting from inhibition of ALA dehydrase is a process occurring among the immature erythrocytes of the bone marrow where the concentration of lead is much higher than it is in peripheral blood (31). In this connection, it is pertinent to note that inhibition of ALA dehydrase in peripheral blood is claimed to persist for as much as four years after cessation of abnormal exposure (55). Perhaps ALA dehydrase inhibition occurs in the marrow where the lead concentration is still high long after the concentration of lead elsewhere has returned to the normal range.

Two groups have recently studied the relative sensitivities of ALA dehydrase inhibition and of ALA excretion as indices of lead exposure (56, 57). Both concluded that inhibition of ALA dehydrase was a more sensitive index of exposure than ALA excretion. In the first of these

two papers, the basis for the conclusion was largely that even among the workers having normal ALA excretions, ALA dehydrase activity was profoundly depressed. In the second paper (57), the same kind of reasoning was applied. Correlation between the degree of ALA dehydrase inhibition and the concentration of lead in the blood was quite poor however. The sensitivity of ALA dehydrase to lead exposure has also been studied in rabbits (58). Again, the high degree of sensitivity of this enzyme to in vivo inhibition was quite apparent. Subcutaneous doses of lead 5 X weekly as low as 0.68 mg/kg caused 50% inhibition within four weeks.

Recently, the inhibitory effect of lead on the erythrocyte membrane Na + K ATPase has been described (59, 60). The degree of enzyme inhibition was not remarkable; nor did it bear any quantitative relationship to other parameters of lead exposure used conventionally.

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